Biophysical Underpinnings of Anisotropic Water Diffusion

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Introduction

All diffusion-tensor magnetic resonance imaging (DTI) studies of nerve, spinal cord white matter, and brain white matter rely on the phenomenon that water diffusion is highly anisotropic in these tissues of the nervous system. The fact that water diffusion is sensitive to the underlying tissue micro-structure provides a unique method of assessing the orientation and integrity of these neural fibres which may be useful in assessing a number of neurological disorders. A basic understanding of the influence of various structural components on anisotropic water diffusion is a pre-requisite for interpreting alterations in diffusion (trace, eigenvalues) and anisotropy as a result of various disease processes or abnormal development. The purpose of this abstract is to characterize the relationship of nuclear magnetic resonance measurements of water diffusion and its anisotropy (i.e. directional dependence) with the underlying micro-structure of neural fibres. A systematic discussion of the possible sources of anisotropy and their evaluation will be presented with an emphasis on model neurological systems both *in-vitro* and *in-vivo*. A comprehensive bibliography of papers before 2002 is provided in my previous review article on this interesting topic (1).

Postulated Sources of Diffusion Anisotropy

Although diffusion taking the path of least resistance along the oriented fibres was an obvious and plausible explanation for the observed anisotropy in neural fibres, little work had been performed to determine the relative contributions of the various structural components to the anisotropy of the water diffusion coefficients. Nonetheless, several possible origins of anisotropy had been postulated. For our purposes and to simplify the discussion, nerves (peripheral, central) and white matter (spinal cord, brain) are all ordered axonal systems that consist of essentially the same primary micro-structural components. The myelin sheath around the axons, the axonal membrane, and the neurofibrils (microtubules, neurofilaments) are three longitudinally-oriented structures that could impart non-random barriers to diffusion (Figure 1) and hence reduce the apparent diffusion coefficient perpendicular to the fibres, ADC($\perp$) [given by the mean of the two smaller eigenvalues of the diffusion tensor, i.e. $(\lambda_2 + \lambda_3)/2$], with respect to the apparent diffusion coefficient parallel, ADC(//) [given by the largest eigenvalue of the diffusion tensor, i.e. λ_1]. Alternatively, some proposed that the diffusion parallel to the length of the axons could be accentuated by axonal transport. Others have suggested that water diffusion, as measured by NMR, could be anisotropic due to local susceptibility-difference-induced gradients in the nerves and white matter. Nerve has proven to be an excellent system to evaluate diffusion anisotropy since it possesses structural components similar to white matter, it can be isolated, it is robust, and when excised it can be oriented readily along the laboratory axes of the diffusion gradients.

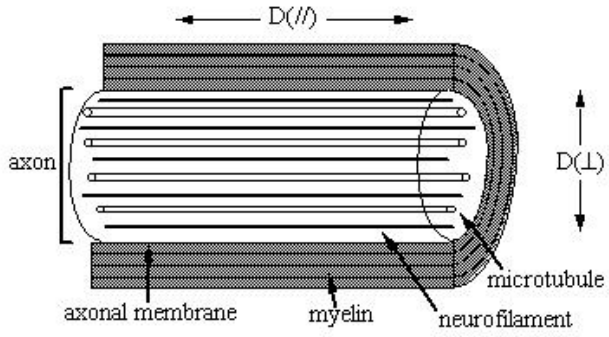

Figure 1 : A simplistic schematic of the longitudinal view of a myelinated axon. Myelin, the axonal membrane, microtubules, and neurofilaments are all longitudinally-oriented structures that could hinder water diffusion perpendicular to the length of the axon and cause the perpendicular diffusion coefficient, D(⊥) to be smaller than the parallel diffusion coefficient, D(//). Other postulated sources of diffusion anisotropy are axonal transport and susceptibility-induced gradients. The narrow extra-cellular space between the numerous packed axons is not illustrated.

Myelin and Axonal Membranes

The preferred, but unproven, hypothesis in the early 1990s for anisotropic water diffusion was the hindrance of perpendicular water diffusion by the myelin sheath encasing the axons. The numerous lipid bilayers of myelin have limited permeability to water and would be expected to hinder diffusion across the fibres but not along the length of the axons where such barriers did not exist. If myelin were the sole source of anisotropy, then it was *expected* that diffusion would be much more isotropic in a normal fibre tract without myelin. In one of the first systematic studies on the underlying source of anisotropy, this was found not to be the case since water diffusion was significantly anisotropic in a normal, intact, non-myelinated olfactory nerve of the garfish (2). The degree of anisotropy in the non-myelinated olfactory nerve [ADC(//)/ADC(⊥) = 3.6] was similar to that observed in the garfish trigeminal nerve myelinated with Schwann cells [ADC(//)/ADC(⊥) = 3.2] and the garfish optic nerve myelinated with oligodendrocytes [ADC(//)/ADC(⊥) = 2.6]. The degree of anisotropy in these excised nerve samples measured at room temperature was quite similar to the anisotropy measured *in-vivo* in humans, lending credibility to the *in-vitro* data. It is important to note that the garfish olfactory nerve is normally non-myelinated and is not a model of demyelination. This study provided the first unequivocal evidence that myelin was not an essential component for anisotropic diffusion in neural fibres. This is not to say that myelin does not play a role in anisotropy, but rather this observation serves to point out that structural features of the axons other than myelin are *sufficient* to give rise to anisotropy and that interpretations of changes in anisotropy with respect to just myelination must be made with caution.

The initial observation of anisotropy in the intact non-myelinated garfish olfactory nerve has subsequently been confirmed in various other models with non-myelinated neural fibres both *in-vitro* and *in-vivo*. Other examples of *non-myelinated* fibres that exhibit significant anisotropic diffusion include the white matter of rat pups prior to histological evidence of myelination (3,4), optic nerve in 2 week old “jimpy” mouse (5), vagus nerve of the rat (6), spinal cord white matter in myelin deficient rat (7), brain white matter in developing mice (8), walking leg nerve of the lobster (1), lamprey spinal cord (9), brain white matter in baby rabbits (10), and the brain white matter of myelin-deficient genetically-engineered shiverer mice (11-13). In addition to animal studies, *in-vivo* human measurements in neonates have shown diffusion anisotropy in non-myelinated fibres of the human brain (14-16). Anisotropic water diffusion in neural fibres must not be regarded as myelin specific.

However, the study on the myelin deficient rat also nicely demonstrated that myelination can *modulate* the degree of anisotropy (7). The anisotropic diffusion ratio (// / ⊥) was ~ 4.5 and ~ 3.5 in the control and myelin deficient white matter, respectively (or A_σ ~ 0.53 and 0.45 with their measure of anisotropy). The anisotropy decreased only by ~ 20% in the myelin deficient rats and signified that the residual structures, namely the membranes of the numerous axons, are sufficient for significant anisotropic diffusion in this model. Similar observations in elegant studies of shiverer mice have been reported (11-13). Relative anisotropy was reduced by 16-25% in various white matter tracts of the shiverer mice although the considerable residual anisotropy confirmed that myelin modulates the degree of anisotropy but that it is not the main factor (11). The reduction in anisotropy was due to an increase in ADC(⊥) with no concomitant change in ADC(//). The treatment of shiverer mice with neural precursor cells created regions with increased diffusion anisotropy that corresponded well with the spatial distribution of donor-derived myelination in individual mouse brains indicated by immunohistochemistry or fluorescence microscopy (12). In a statistical parametric mapping study of shiverer mice, there was a general increase in parallel and perpendicular diffusivities (and trace ADC); these individual diffusion parameters seemed to be more sensitive to dysmyelination than the derived fractional anisotropy (13). This agrees with the demonstration in a mouse optic nerve after retinal ischemia that decreases of ADC(//) and increases of ADC(⊥) may be specific markers for the identification of axonal degeneration versus myelin loss, respectively, whereas both pathologies would result in non-specific reduced anisotropy (17).

In general though, a quantitative or even qualitative determination of the relative importance of axonal membranes and myelin for anisotropy in a particular fibre tract is difficult to assess. Direct comparisons of the degree of anisotropy between unique fibres with different axon diameters, degree of myelination, and fibre packing density are fraught with difficulty. Various diffusion indices such as ADC(⊥), ADC(//), and anisotropy index have correlated with numerous histological parameters of axon morphometry, primarily axon density, in normal myelinated, rat cervical spinal cord (18).

As we will see below, the other potential contributors (neurofibrils, fast axonal transport, susceptibility) to anisotropy are not significant.

Neurofibrils and Fast Axonal Transport

The complex and dense three-dimensional cytoskeleton of axons is mainly composed of longitudinal-oriented, cylindrical neurofibrils, namely microtubules and neurofilaments, which are inter-connected by small microfilaments. These structures could presumably cause anisotropic diffusion if the small, but numerous, neurofibrils presented sufficient physical barriers to hinder perpendicular water diffusion to a greater extent than parallel. In addition, fast axonal transport is intimately linked to the presence of microtubules since cellular organelles are transported along the microtubule tracks.

The role of microtubules and fast axonal transport in anisotropic diffusion was evaluated in excised myelinated and non-myelinated nerves of the garfish that were treated with vinblastine that is known to depolymerize microtubules and inhibit fast axonal transport (2). Anisotropy was

preserved in all three types of nerve treated with vinblastine suggesting that microtubules themselves and the fast axonal transport they facilitate do not contribute to anisotropy.

This earlier study did not assess the role of the numerous longitudinal-oriented neurofilaments, the primary structural component of the axoplasm. The influence of the neurofilamentary cytoskeleton on water mobility was evaluated by making measurements in axoplasm with minimal interference from membranes (19). The isolated giant axon from squid was used because it can provide an axoplasmic space (diameter ~ 200-1000 μm) whose dimension is much greater than the one-dimensional, root-mean-square displacement (~11 μm) of a water molecule randomly diffusing over typical diffusion times used in NMR studies (~ 30 ms). The diffusion coefficients of water parallel and perpendicular to the long axis of the squid giant axon at 20°C were 1.6×10^{-3} mm^2 s^{-1} and 1.3×10^{-3} mm^2 s^{-1}, respectively, which yielded an anisotropic diffusion ratio (// / ⊥) of only 1.2, which is nearly isotropic. This experimental measure of anisotropy matched Monte Carlo computer simulations of randomly diffusing particles in a regular, hexagonal array of circular barriers whose size (10 nm) and spacing (20-60 nm) were chosen to simulate the neurofilamentary lattice (19). Two important findings resulted from this work. First, the neurofilaments do not have a significant role in producing diffusion anisotropy within the axon and thus pointed towards the importance of membranes in fulfilling the role as the primary determinant of the observed anisotropy in neural fibres. Second, water diffusion in pure axoplasm (i.e. intra-cellular space of axons) is rapid and is ~70-80% of that in pure water at 20°C (ADC ~ 2×10^{-3} mm^2 s^{-1}).

Our work on the excised and isolated squid giant axon was confirmed by diffusion micro-imaging of the non-myelinated lamprey spinal cord which consists of variable axon diameters (9). In very large axons whose diameter is much greater than the root-mean-square displacement of diffusing water molecules, the parallel and perpendicular ADCs were identical (and fast ~0.98 $\times 10^{-3}$ mm^2 s^{-1}) indicating isotropic diffusion in the absence of interaction with membranes. In contrast, water diffusion was anisotropic in white matter regions with multiple packed axons; however, ADC(//) was the same as the single axon case whereas the ADC(⊥) was reduced markedly and varied inversely with the number of axons (membranes) over a fixed distance.

Susceptibility

Anisotropic water diffusion, as measured by NMR, could result from local susceptibility-difference-induced gradients in the nerves and white matter and its first evaluation was performed on excised porcine spinal cord at 4.7T (20). By varying the orientation of the fibre tracts parallel or perpendicular to the static magnetic field (B_o), the background gradients could be minimized or maximized, respectively. The ADCs parallel or perpendicular to the fibres measured with the standard PGSE diffusion sequence were independent of the fibre orientation relative to B_o and hence the induced gradients do not play a role in the anisotropy of diffusion in white matter. They also used a bipolar gradient pulse sequence to eliminate the effect of the background gradients on the ADC values. The ADC and anisotropy independence on susceptibility-induced gradients was confirmed subsequently in four different excised nerves from garfish and frog at 2.35T (21) and in human brain white matter in-vivo at 1.5T (22).

Other Issues

Numerous studies on restricted diffusion, dependence on diffusion time, multiple compartments, pathology, development, and computer modeling are not outlined here but some will be covered very briefly as they pertain to this discussion on the underlying causes of anisotropic diffusion.

Conclusions

Although the interpretation of water diffusion in a complex biological tissue such as nerve and white matter is not trivial, numerous studies over the last 15 years have provided a better understanding of the relationship between diffusion and the underlying micro-structural components. Evidence suggests that anisotropic water diffusion in neural fibres is mainly due to the dense packing of axons and that it is the inherent axonal membranes that hinder water diffusion significantly perpendicular to the long axis of the fibres relative to the preferential parallel direction. Hence, axonal membranes are concluded to be the primary determinant of anisotropy although myelination certainly modulates the degree of anisotropy in a given fibre tract. Nonetheless, one should exercise caution in interpreting differences of anisotropy.

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